

| Rank | Scores | | Sequence | (Position) |
|------|--------|-------|------------------------|------------|
| | Model | Local | | |
| F1 | 6 | 1 | GGTAACGGCTCACCTAGGC | (246) |
| F2 | 7 | 1 | CCAGATGGGATTAGCTAGTAGG | (221) |
| F3 | 8 | 1 | GACCAGGGCTACACACGTG | (1196) |
| F4 | 8 | 1 | GTAACGGCTCACCTAGGCG | (247) |
| F5 | 9 | 1 | GGGTAACGGCTCACCTAGG | (245) |
| F6 | 10 | 1 | TAACGGCTCACCTAGGCCA | (248) |
| F7 | 10 | 1 | CCCAGATGGGATTAGCTAGT | (220) |
| F8 | 11 | 1 | AAACGATGTCGACTTGGAG | (797) |
| F9 | 11 | 1 | AGGCGACGATCCCTAGCTG | (261) |
| F10 | 11 | 1 | TAAACGATGTCGACTTGGAG | (796) |
| F11 | 12 | 1 | GAAGTCCGGAATCGCTAGTAAT | (1312) |
| F12 | 13 | 1 | AAGACCAAAGAGGGGGACC | (176) |

| Rank | Scores | | Sequence | (Position) |
|------|--------|-------|-----------------------|------------|
| | Model | Local | | |
| R1 | 5 | 1 | CCGTTACCCACCTACTAGC | (253) |
| R2 | 6 | 1 | TAGGGATCGTCGCCTAGGT | (275) |
| R3 | 7 | 1 | GCCGTTACCCACCTACTAG | (254) |
| R4 | 8 | 1 | CTAGGGATCGTCGCCTAGG | (276) |
| R5 | 8 | 1 | CGCCATTGTAGCACGTGTG | (1225) |
| R6 | 9 | 1 | AGGGATCGTCGCCTAGGTG | (274) |
| R7 | 9 | 1 | CGTTACCCACCTACTAGCT | (252) |
| R8 | 10 | 1 | CGTTACCCACCTACTAGCTA | (252) |
| R9 | 11 | 1 | CTGATCCACGATTACTAGCG | (1342) |
| R10 | 11 | 1 | TCCTCTCAGACCAGCTAGG | (290) |
| R11 | 11 | 1 | AAGGGCACAACTCCAAGT | (826) |
| R12 | 12 | 1 | TCATCCTCTCAGACCAGCTAG | (293) |

Figure 21. Primer Selection Search Results

III Hybridization Temperature Calculation

Hybridization is usually few degrees lower than the melting temperature. There are many published models to calculate the melting temperature. Most of them are based on the following simplified equation:

$$T_m = 81.5 + 16.6 * \log_{10}(C_{Na}) + 41 * f_{GC} - 600/n_{total}$$